THE EFFECT OF MYCORRHIZAL INOCULATION ON THE GROWTH AND ROOT RESPIRATION OF SCOTCH PINE SEEDLINGS

Peitsa Mikola

Department of Silviculture
University of Helsinki

1. Introduction

The value of different species of fungi as symbionts of forest trees is a crucial problem of mycorrhizal research in forestry. This problem could perhaps be solved by long-term field experiments. Such experiments, however, are not possible in countries where practically all soils are inhabited by an indigenous population of mycorrhizal fungi. Instead, respiratory studies with the Warburg technique may reveal some differences between different fungal species.

The Warburg technique has been successfully adopted for studies on the respiration of mycorrhizal and non-mycorrhizal roots. Thus, for instance, the much higher respiration rate of mycorrhizal roots has been demonstrated (ROUTIEN & DAWSON 1943), and the correlation between respiration and salt absorption of mycorrhizae has been extensively studied (cf. HARLEY 1959, pp. 93-97). The aging of mycorrhizae (MIKOLA 1953) and the effect of biocides on them (VOIGT 1954) have also been studied by manometric technique.

Below are reported some results of an experiment in which uninoculated pine seedlings and seedlings, which had been inoculated, in the first case, with a pure culture and, in the second, with a mixed population, were grown under field conditions.

2. The Experiment

Previous experiments (MIKOLA 1965) had shown that non-mycorrhizal pine seedlings, grown on autoclaved peat in open pots, remained non-mycorrhizal at least to the end of the first summer, whereas, in the case of seedlings inoculated with pure cultures of an ectendotrophic mycorrhizal fungus, all the short roots were invariably typical ectendotrophic mycorrhizae as early as a few weeks after inoculation. Accordingly, this particular fungus had proved suitable for inoculation experiments and was also used in the following experiment. The strain, E-57, had been isolated from an ectotrophic spruce mycorrhiza in a nursery in the autumn of 1962; in synthesis experiments, however, it always formed ectendotrophic mycorrhizae with pine (cf. MIKOLA 1965, pp. 14-18).

For comparison, suspension of forest humus from a pine stand was also used as inoculum, representing the natural fungal population of pine forests.

Seedlings were raised in peat pots ("Jiffy pots") of 125 ml capacity. Milled peat was autoclaved and put in the pots which were then kept on autoclaved sand in metal boxes. Five to ten pine seeds were sown per pot on May 17, 1964. Before sowing, the pots received a moderate fertilization (10 ml per pot of solution containing 10 g of dolomitic lime stone, 0.8 g of K_2SO_4, 0.5 g of double superphosphate, 0.5 g of Ca-NH_4 salpetre, and 0.1 g of mixture of micro nutrients per litre).

Inoculation took place on June 25 and 26, i.e. approximately one month after germination. At the same time the pots were thinned by leaving three seedlings per pot. Pure culture inoculation was performed by cutting a piece of mycelium (approx. 1 cm^2) from agar plate and burying it in the peat between the three seedlings. For forest humus inoculation, humus was homogenized in water and 2 ml of suspension per pot was used.

The pots were transplanted in the field on Aug. 4 and 5. A probably non-mycorrhizal soil was available on a field where peat had been removed for fuel and horticultural use. The area had originally been a treeless bog with a 3 m thick peat layer. After drainage peat had been harvested for years and the last yields were taken in the early summer of 1964, when boulders of the subsoil became visible and the field, which still supported a half metre thick layer of peat, on the average, was abandoned. The experiment was established as a 3 x 3 Latin square, each plot containing 120 pots at 1 m spacing.

In the following spring (June 4, 1965) the plots were further divided into two subplots, with 60 planting spots in each, and one of the subplots was fertilized by giving 20 g of fertilizer mixture (10 % of N, 12 % P_2O_5 and 6 % of K_2O) per planting spot.

Samples for microscopic examination of short roots and measurement of root respiration were taken repeatedly in the following summers. In the autumn of 1966 the length of top shoots was measured and top shoot needles were sampled for chemical analyses.

3. Results

31. Mycorrhizal infection

As was shown by previous experiments (MIKOLA 1965), mycorrhizal infection takes place very rapidly after inoculation with pure culture of the ectendotrophic fungus, and one of the first signs of infection is an increase of dichotomous branching of the short roots. This was also confirmed by the present experiment. At the time of planting (Aug. 6, 1964) short roots of the seedlings which had been inoculated with E-57, had an ectendotrophically mycorrhizal structure, whereas in those seedlings inoculated with humus suspension mycorrhizal infection could not yet be observed with certainty. A month later a survey revealed the following percentages of dichotomous short root tips:

Control	26-52 %
Inoculated with E-57	80-82 %
Inoculated with humus	57-67 %

Repeated microscopic examination revealed that the control seedlings remained non-mycorrhizal a relatively short time only. In the early summer of 1965 no mycorrhizal infection was detected but in the late summer typical ectotrophic mycorrhizae with a mantle and Hartig net were observed. In the summer of 1966 all the control seedlings were mycorrhizal. Since there were hardly any mycorrhizal fungi in the soil, the infection had most probably taken place through spores from surrounding forests.

The seedlings which were inoculated with E-57 retained the ectendotrophic type of infection for at least two years, i.e. until the autumn of 1966. In 1965 it was the only type on those seedlings; in the late summer of 1966, however, ectotrophic mycorrhizae were also observed, although the ectendotrophic one was still the predominant type.

Inoculation with humus suspension was also effective, although the infection took place somewhat more slowly than in seedlings inoculated with E-57. In the early summer of 1965, when all the control seedlings were still non-mycorrhizal, mycorrhizae were common in the seedlings inoculated with humus. Later on these seedlings were characterized by a great diversity of mycorrhizae, as could be expected from an inoculation with a mixed population. Thus, the thickness and colour of the mantle varied, and there were also tuberous C mycorrhizae; the ectendotrophic type characteristic of inoculation with E-57 was, however, absent.

The influence of fertilization was also easily detected. In the late summer of 1965 and early summer of 1966 all the fertilized seedlings showed a much weaker development of mycorrhizae than the unfertilized ones. This is in good agreement with the results of BJÖRKMAN (1942, 1949) who also observed the retarding effect of strong fertilization on the development of mycorrhizae. In the late summer of 1966 the

concentration of the fertilizers had decreased so much that no difference could be observed between fertilized and unfertilized seedlings.

32. Root respiration

A Warburg apparatus (Model V85 of B.Braun, Melsungen) was used for measurements of root respiration. The reaction vessels contained either pieces of long roots with attached short roots (Aug. 1965) or short roots only (other measurements), the dry weight ranging from 5 to 15 mg. Each treatment was represented by two (Aug. 1965) or three (other measurements) replications.

The first measurements were conducted on June 4, 1965, i.e. simultaneously with the fertilization. Another corresponding experiment, when root respiration was measured repeatedly in the same summer as inoculation (with E-57) was performed, showed that two months after inoculation the root respiration was, on the average, 50 % higher in the inoculated than in the uninoculated seedlings.

Measurements for two summers (Table 1) showed that the root respiration of inoculated seedlings was consistently greater than that of uninoculated ones (Fig. 1). In the second summer, however, the difference was smaller, as could be expected, since by then mycorrhizal infection had also taken place in the uninoculated seedlings. The natural mycorrhizal population of a forest soil (inoculation with humus suspension) increased root respiration more than the pure culture inoculation with the ectendotrophic fungus E-57. This may be due to the fact, that the mycorrhizae of humus-inoculated seedlings had a thicker mantle and more outside mycelia, whereas in the ectendotrophic mycorrhizae the fungus was mainly confined in the Hartig net and cortical cells. Root respiration of the seedlings inoculated with humus suspension showed greater variation, and differences between replications were also relatively great, the reason for which may be the fact that various types of mycorrhizae and apparently several species of fungi were involved. In the other treatments (control and E-57 inoculation) differences between replications were remarkably small.

Table 1. Oxygen uptake of pine short roots (µl/mg/h).

Inoculation	Fertili-zation	June 4 1965	Aug.20 1965	Aug.25 1965	June 4 1966	Aug.16 1966	Aug.29 1966
- (control)	-	1.86	0.69	0.58	2.61	0.39	0.53
- "	+		1.05	0.68	3.00	1.09	1.51
E-57 p.c.	-	2.02	0.95	1.48	2.85	0.61	0.56
- " -	+		0.93	1.01	1.90	1.79	0.97
Humus susp.	-		2.47	1.63	3.06	1.08	0.64
- " -	+		0.95	0.97	1.77	1.47	1.45

The effect of fertilization was interesting (Fig. 2). As regards the controls the root respiration rate of fertilized seedlings was consistently higher than that of unfertilized seedlings, whereas with the inoculated seedlings the opposite was at first the case. In the late summer of 1966, however, the discrepancy disappeared and the respiration rate of the fertilized seedlings exceeded that of the unfertilized ones in the case of the inoculated seedlings as well. This is in good agreement with the observations on mycorrhizal development and the results of BJÖRKMAN (1942, 1949). Fertilization increased the root respiration of control seedlings, whereas in the inoculated seedlings fertilization retarded mycorrhizal development to such a degree that root respiration was also reduced. In the late summer of 1966, however, the concentration of fertilizers had decreased to a level favourable for mycorrhizal development and, therefore, the root respiration of the fertilized seedlings exceeded that of the unfertilized ones.

33. The growth of seedlings

The average length of top shoots in 1966 gives a good picture of the relative growth of the seedlings under different treatments (Table 2). Although differences in mycorrhizal relations had already been greatly levelled off by outside infection, clear differences can be noticed particularly in the

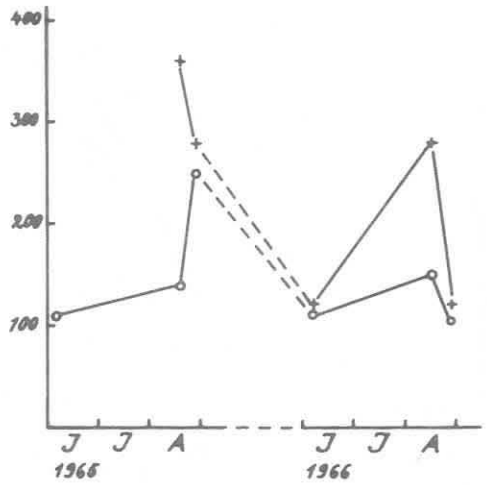

Fig. 1. Relative O_2 uptake of unfertilized seedlings.

100 = O_2 uptake of control seedlings

o Inoculated with E-57 pure culture
+ Inoculated with humus suspension

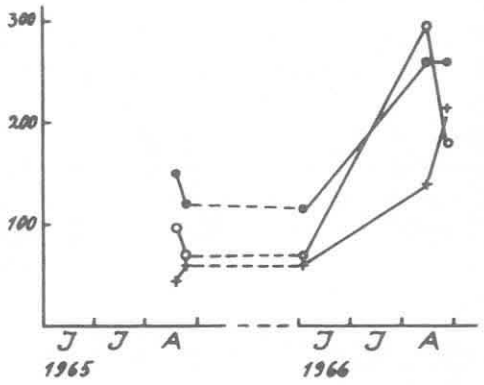

Fig. 2. Relative O_2 uptake of fertilized seedlings.

100 = O_2 uptake of unfertilized seedlings

- • Not inoculated
- o Inoculated with E-57 pure culture
- \+ Inoculated with humus suspension

growth of unfertilized seedlings. The difference between the control seedlings and those inoculated with forest humus may be taken as an indication of the favourable effect of mycorrhizal inoculation. Although the control seedlings were already mycorrhizal by 1966, the infection had taken place considerably earlier in the inoculated seedlings which were, therefore, still in advance of them in that year.

Table 2. Average lenght (cm) of the top shoots in 1966.

Inoculation	Not fertilized		Fertilized	
	Individual plots	Mean	Individual plots	Mean
Control	8.52 9.07 5.12	7.59	11.19 12.10 13.18	12.20
E-57	7.59 4.96 3.93	5.51	15.43 14.14 12.57	14.05
Humus	11.96 10.14 8.20	10.12	12.64 13.19 12.75	12.86

Significance of differences between means.

Difference	t	
	Not fertilized	Fertilized
E57-Control	-4.8105^{xxx}	4.3869^{xxx}
Humus-Control	6.0673^{xxx}	1.5939

The unfertilized seedlings inoculated with E-57 grew more poorly in 1966 than the controls. This does not indicate, however, that E-57 was altogether harmful to the seedlings, since by 1966 the control seedlings were also mycorrhizal. It shows only that under the prevailing conditions E-57 was

inferior as a symbiont to the fungi in the humus inoculum or those spread to the control seedlings from the surroundings. Corresponding results were obtained by RAYNER (1934) and LEVISOHN (1954) who regard "the haustorial type of infection" as inferior to the ectotrophic mycorrhiza (cf. MIKOLA 1965, p.50). On the other hand, according to experiments of LAIHO (1965, pp.20-23), seedlings inoculated with E-57 grew better than uninoculated controls under semiaseptic conditions.

The fertilized seedlings show quite a different picture, differences between the different groups being relatively small. This fact can be explained, since strong fertilization retarded mycorrhizal development, and, by the time the concentration of fertilizers had decreased in 1966, the uninoculated seedlings had also become mycorrhizal. It is important to notice, however, that on the fertilized plots the seedlings inoculated with E-57 grew best, the difference being statistically significant (Table 2).

Table 3. Concentration of N, K_2O, and P_2O_5 in top shoot needles in the autumn of 1966, per cent of D.W.

Inoculation	Unfertilized seedlings			Fertilized seedlings		
	N	K_2O	P_2O_5	N	K_2O	P_2O_5
Control	2.41	0.48	0.17	1.92	0.63	0.40
E-57	2.23	0.50	0.20	1.85	0.68	0.40
Humus	2.18	0.43	0.19	1.62	0.56	0.41

No clear influence of inoculation on the chemical composition of needles could be observed in 1966 (Table 3). The effect of fertilization was all the more pronounced. The nitrogen concentration was higher in the unfertilized than in the fertilized seedlings, indicating that nitrogen was not a limiting soil factor. On the other hand, the phosphorus concentration in the unfertilized seedlings indicated a severe lack of available phosphorus in the soil and, therefore, fertilized seedlings had more than twice as high a phosphorus

content. The potassium content of the needles was also considerably higher in the fertilized seedlings, the concentration of the unfertilized seedlings showing a slight potassium deficiency (cf. GUSSONE 1964, p.26, for instance).

4. Discussion

Root respiration alone does not prove that one fungal species is more beneficial than another, since even purely parasitic fungi may cause an intense gas exchange in the roots. Measurements of root respiration can, however, complement the information that has been obtained with other methods.

As a whole, the above experiment shows that under Finnish conditions non-mycorrhizal seedlings can be grown in the field a short time only. Even on the bottom of a peat bog non-mycorrhizal seedlings receive mycorrhizal infection very repidly. The spread of spores of mycorrhizal fungi through the air must be very effective.

The value of the ectendotrophic fungus E-57 as a symbiont of pine can not be judged solely on the above single experiment. This experiment shows, however, that the problem may be quite complicated. Field experiment with pure culture inoculation would be most desirable in areas where the risk of outside contamination is minimal.

5. References

BJÖRKMAN, E. 1942. Die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. - Symb. Bot. Upsal. 6.

BJÖRKMAN, E. 1949. The ecological significance of the ectotrophic mycorrhizal association in forest trees. - Sv. Bot. Tidskr. 43.

GUSSONE, H.A. 1964. Faustzahlen für Düngung im Walde. - BLV. München.

HARLEY, J.L. 1959. The biology of mycorrhiza. - Leonard Hill Ltd. London.

LAIHO, O. 1965. Further studies on the ectendotrophic mycorrhiza. - Acta Forest. Fenn. 79.

LEVISOHN, I. 1954. Aberrant root infection in pine and spruce. - New Phyt. 53.

MIKOLA, P. 1953. Mykoritsain merkitys puiden ravinnonotossa. - Luonnon Tutkija 57.

MIKOLA, P. 1965. Studies on the ectendotrophic mycorrhiza of pine. - Acta Forest. Fenn. 79.

RAYNER, M.C. 1934. Mycorrhiza in relation to forestry. I. Researches on the genus Pinus, with an account of experimental work in a selected area. - Forestry 8.

ROUTIEN, J.B. & DAWSON, R.F. 1943. Some interrelationships of growth, salt absorption, respiration, and mycorrhizal development in Pinus echinata Mill. - Amer. J. Bot. 30.

VOIGT, G.K. 1954. The effect of fungicides, herbicides, and insecticides on the accumulation of phosphorus by Pinus radiata as determined by the use of P^{32}. - Agronomy Jour.